Copanlisib synergies with conventional and targeted agents including venetoclax in B- and T-cell lymphoma models

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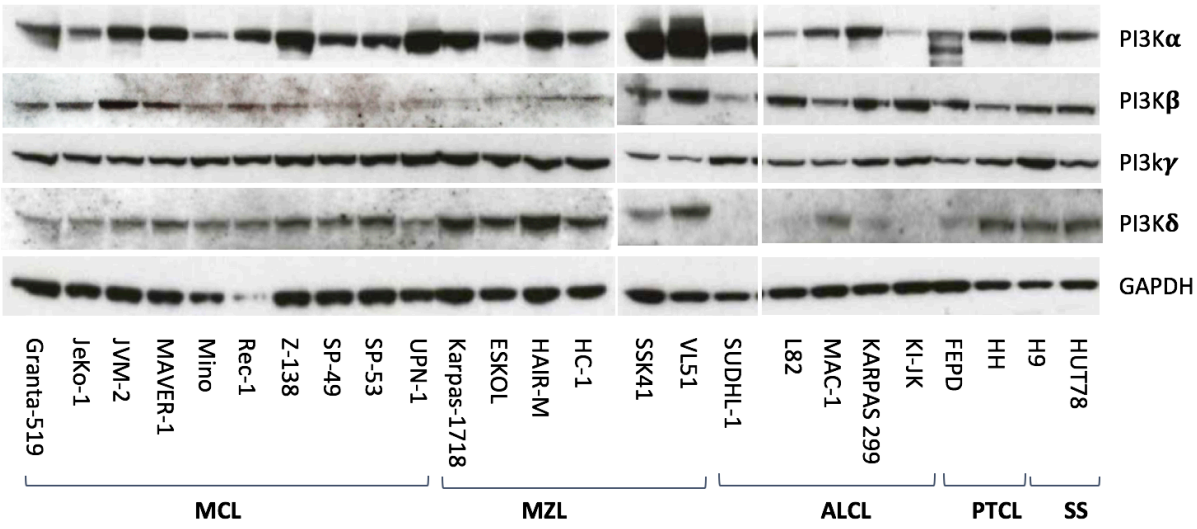
Statement of equal author contribution

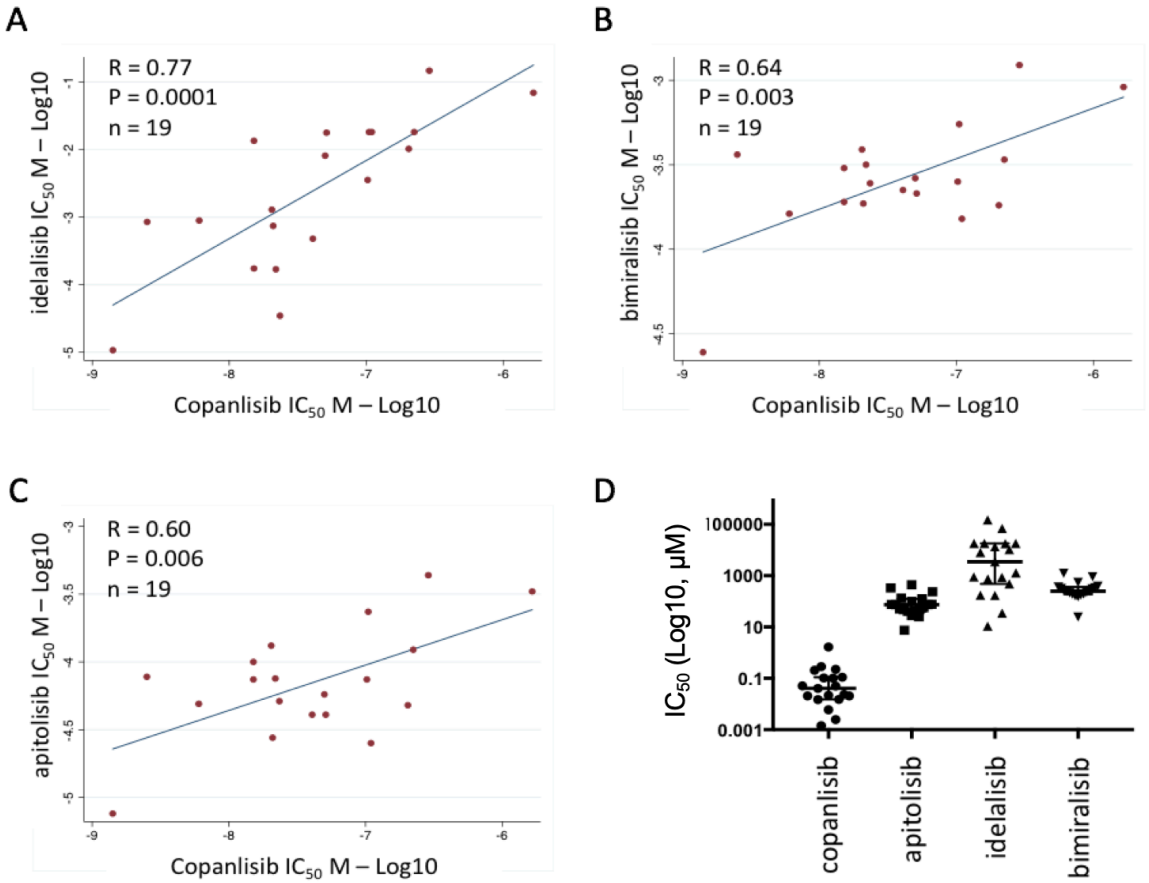
CT and ML contributed equally to this study.

SUPPLEMENTARY MATERIAL

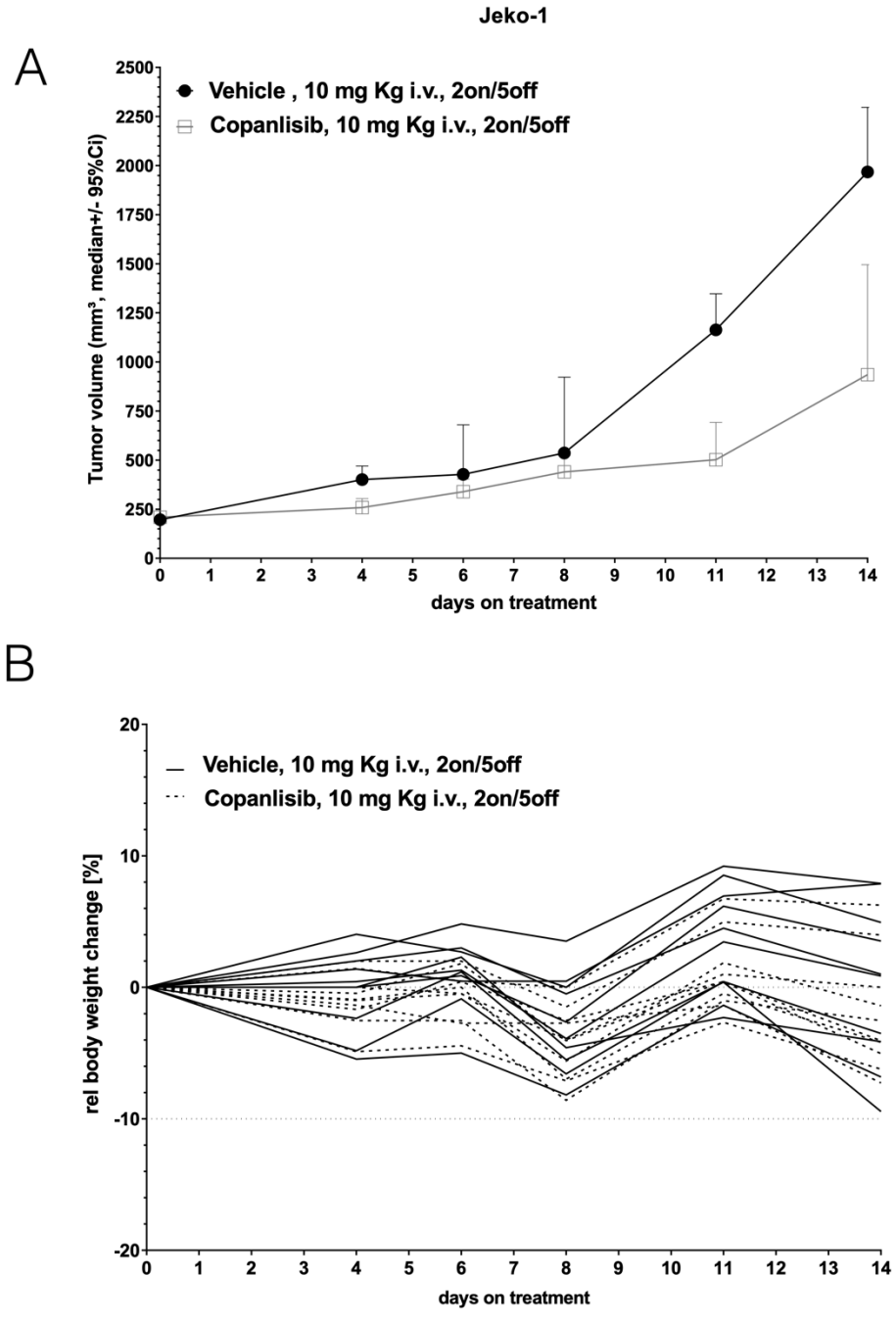
Supplementary Figure 1. **Protein levels of PI3K isoforms in lymphoma cell lines.** Ten MCL, six MZL, five ALCL, two PTCL and two SS cell lines were analyzed for baseline PI3K isoforms protein expression. GAPDH was used as loading control.



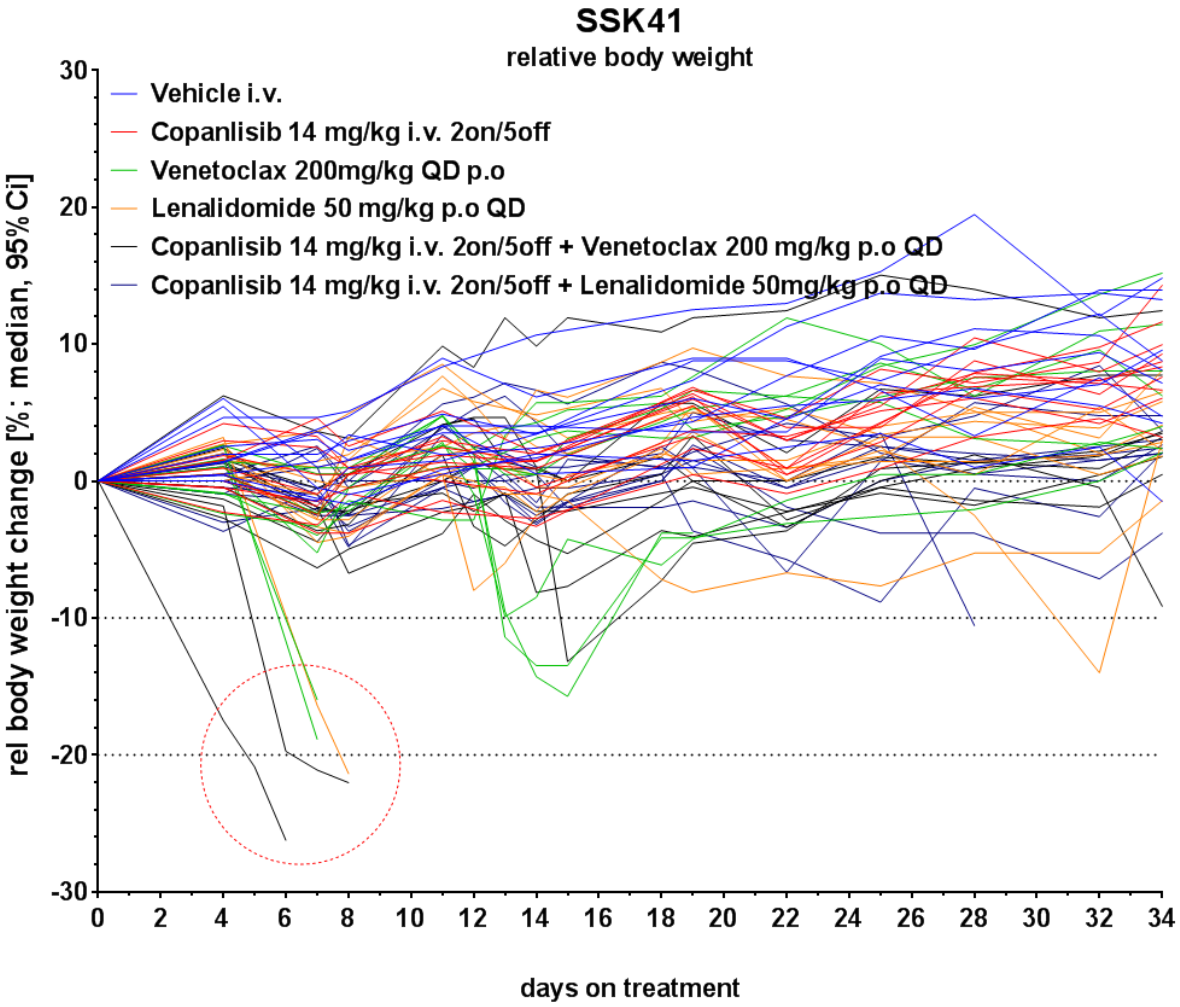
Supplementary Figure 2. The *in vitro* antitumor activity of copanlisib and other PI3K inhibitors are correlated across B-cell lymphoma lines. Correlation between copanlisib and idelalisib (A), bimiralisib (B), apitolisib (C) activity (IC50s), respectively. Y-axis, Log10 IC50 values (M). D) IC50s comparison in a panel of 19 cell lines. The line in the middle represents the median IC50 with 95% of CI. Y-axis, Log10 IC50 values (μM). Each point represents one cell line with the respective IC50. R, correlation coefficient; P, p-value.



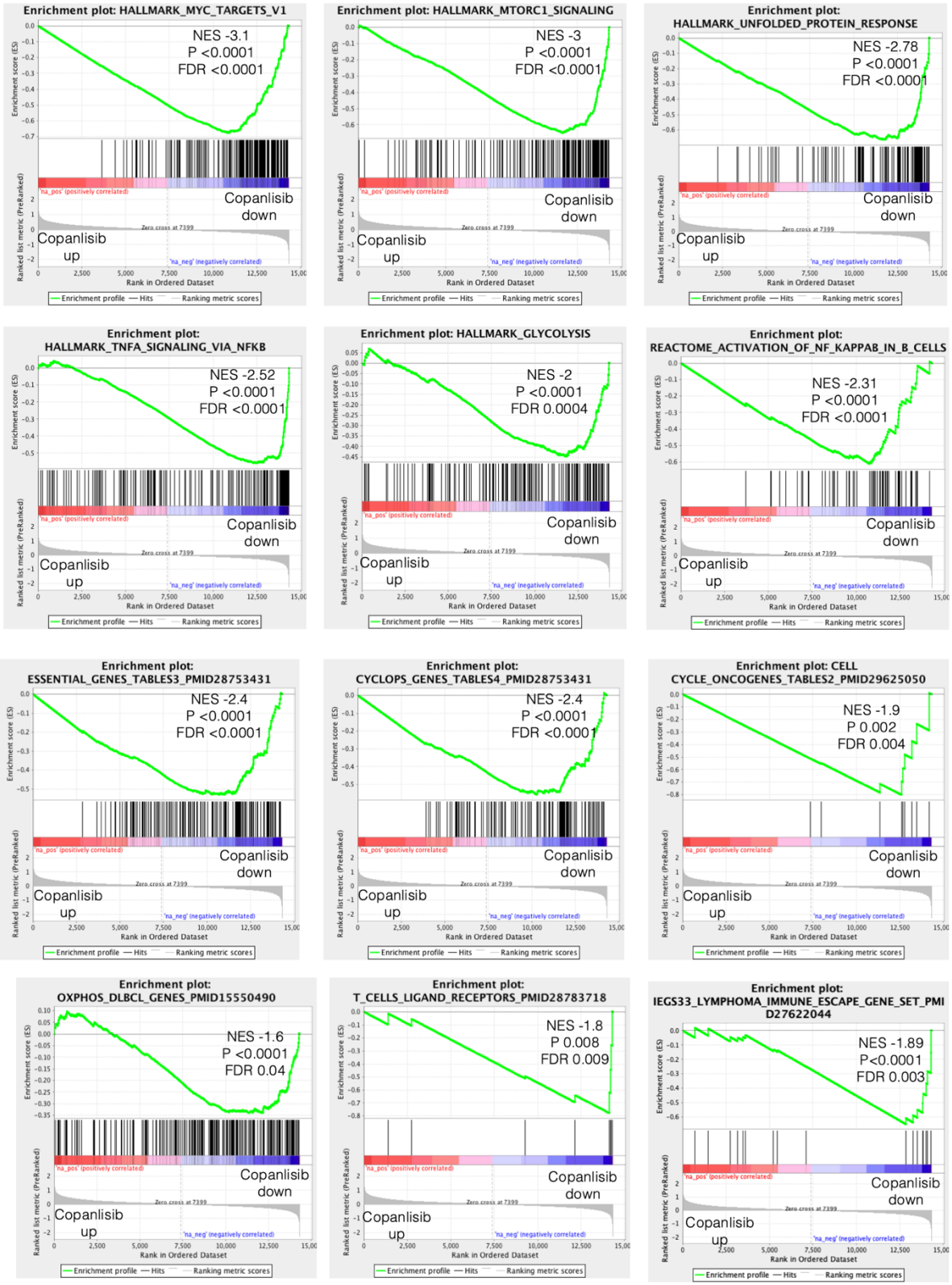
Supplementary Figure 3. Copanlisib has *in vivo* anti-tumor activity in the MCL JEKO1 xenograft model. A) Mice were treated with vehicle (i.v.) or copanlisib (10 mg Kg i.v., two days on/five days off). Lines show median values per timepoint with the corresponding upper interquartile range. Black line, vehicle; gray line, copanlisib). Y-axis, tumor volume in mm^3. X-axis, days of treatment. B) Corresponding relative body weight changes (black line continuous line, vehicle; dashed line, copanlisib).



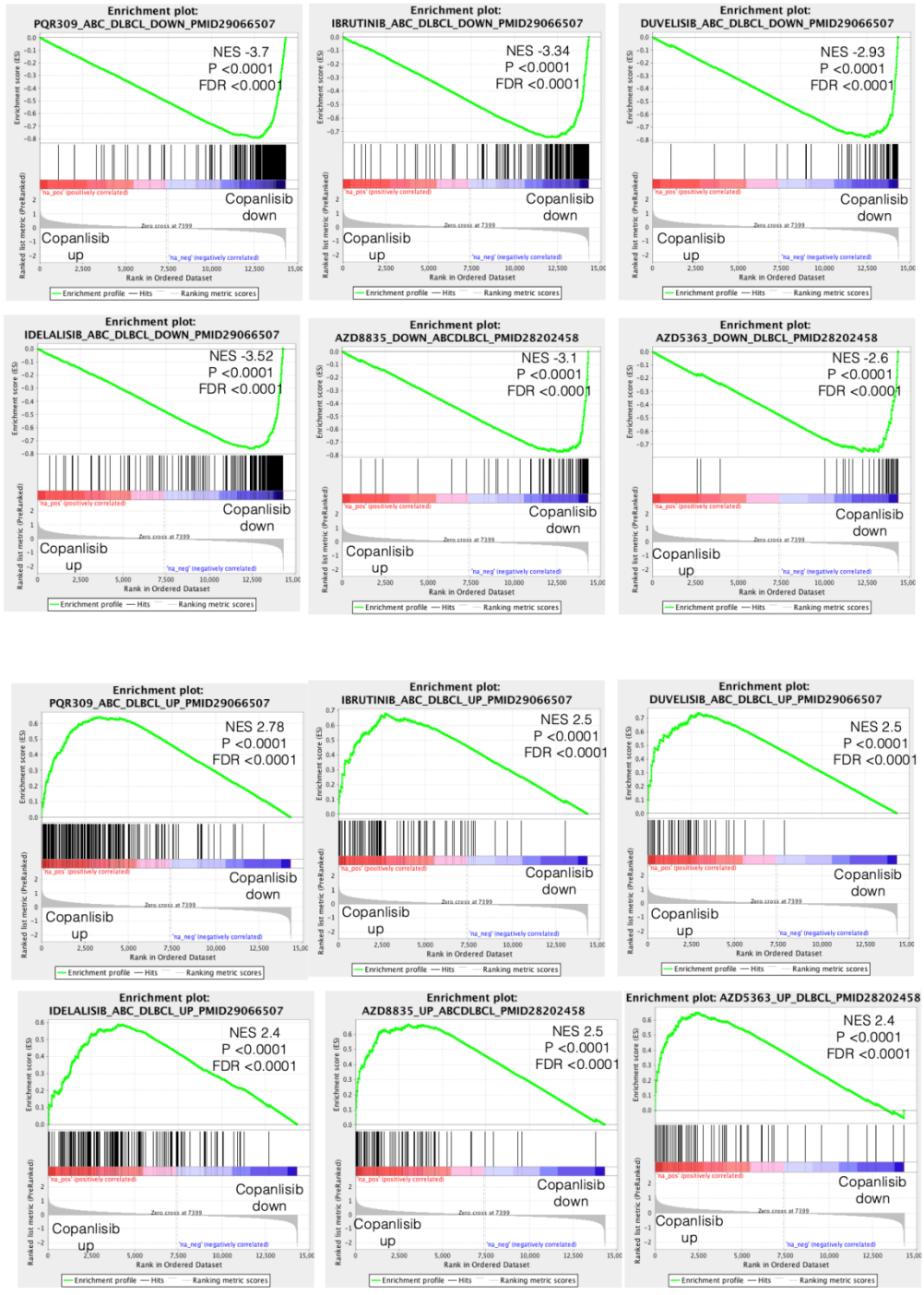
Supplementary Figure 4. Relative body weight changes in mice treated with copanlisib-containing combinations in the *in vivo* experiment shown in Figure 1C.
Mice engrafted with the MZL SSK41 cell line were treated with vehicle (i.v.), copanlisib (14 mg Kg i.v., two days on/five days off), venetoclax (200 mg/Kg, p.o., once per day), lenalidomide (50 mg/Kg, p.o., once per day), copanlisib plus venetoclax (same doses as the single agents) or copanlisib plus lenalidomide (same doses as the single agents). The dotted circle line highlights the weight loss in individual mice treated with lenalidomide and venetoclax monotherapies and in combination.



Supplementary Figure 5. Transcriptome changes in the HAIR-M MZL cell line exposed to copanlisib. Representative GSEA plots illustrating the transcriptional expression signature enrichment in genes downregulated by copanlisib. Green line, enrichment score; bars in the middle portion of the plots show where the members of the gene set appear in the ranked list of genes. Positive or negative ranking metric indicate correlation or inverse correlation with the profile, respectively. FDR, false discovery rate; NES, normalised enrichment score.



Supplementary Figure 6. Transcriptome changes in lymphoma cell lines treated with copanlisib or with other small molecules. Representative GSEA plots illustrating the gene expression signatures of other small molecules in the HAIR-M MZL cell line exposed to copanlisib. Green line, enrichment score; bars in the middle portion of the plots show where the members of the gene set appear in the ranked list of genes. Positive or negative ranking metric indicate correlation or inverse correlation with the profile, respectively. FDR, false discovery rate; NES, normalised enrichment score.



Supplementary Table 1. IC50 values [M] for copanlisib and additional 17 compounds in 25 lymphoma cell lines. Values calculated after 72 h exposure.

Supplementary Table 2. *In vivo* combinations in the MZL SSK41. Differences in tumor volumes were calculated using the Wilcoxon rank-sum test. P-values < 0.05 are highlighted in red.

	copanlisib	lenalidomide	venetoclax	copanlisib, lenalidomide			copanlisib, venetoclax		
day	vs ctr	vs ctr	vs ctr	vs ctr	vs copanlisib	vs lenalidomide	vs ctr	vs copanlisib	vs venetoclax
7	0.0126	0.0412	0.033	0.0025	0.0126	0.0864	0.0045	0.0045	0.0209
10	0.0156	0.253	0.0621	0.0032	0.0696	0.0143	0.0077	0.0506	0.1152
13	0.0025	0.009	0.0263	0.0015	0.0012	0.0019	0.0034	0.0077	0.0117
14	0.0065	0.05	0.1432	0.0009	0.0052	0.0011	0.0025	0.0077	0.0026
15	0.0284	0.0864	0.2416	0.0009	0.0003	0.0003	0.01	0.041	0.0026
16	0.0413	0.1914	0.4945	0.0012	0.0007	0.0019	0.0164	0.1097	0.0206
17	0.0233	0.0412	0.9223	0.0011	0.0002	0.0009	0.0164	0.041	0.0026
18	0.1509	0.1651	0.1432	0.0019	0.0002	0.0031	0.0756	0.0914	0.0038

Supplementary Table 3. Combination indexes for copanlisib combined with additional 17 compounds in 25 lymphoma cell lines, respectively. Values calculated after 72 h exposure.

Supplementary Table 4. Transcriptome changes after copanlisib exposure in the MZL cell line HAIR-M: differentially expressed transcripts as identified with limma analysis (A) and GSEA functional annotation of the copanlisib-induced changes in up- (B) and down-regulated (C) genes.